Supplemental Material

CAR-T cell manufacturing and CD107a degranulation assay

Equal number of T-cells (1.5-2 x10^6/ml/group) were concomitantly treated with 4HPCP (2.5µM) ± NAC (5mM) for 48H and then washed with media once to remove the dead cells and wash-off the 4HPCP-NAC from the culture media. Washed cell numbers and size were measured using Beckman Coulter Counter and equal number of live cells from each treatment group were stimulated with CD3/28 beads (3:1). CART-19 retroviral particle (3:1) were added 24H after bead stimulation for CAR-T cell manufacturing. After 7-days, BDS were removed and the cell culture were continued till the cell size reaches to 300-350 fl (around day 11-13) in each group to perform the CD107 degranulation assay.

Briefly, manufactured CAR-T cells from each treatment group were incubated with target cells (Nalm-6) at a 1:1 ratio in T cell media in 48-well tissue culture plate. Anti-CD107a (Biolegend; #328612), anti-CD28 (BD Biosciences; #555726), anti-CD49d (BD Biosciences; #555501) antibodies cocktail were added to the culture and incubated for one hour at 37°C. After one-hour intracellular protein transport was halted by addition of GolgiStop (BD Biosciences; #554724) and cells were incubated for an additional three hours at 37°C. After four hours of total incubation, cells were harvested and stained for CD-107a, CD3 (Biolegend; #300328) detection. Cells were then fixed and permeabilized using FIX & PERM Cell Fixation & Cell Permeabilization Kit (Invitrogen, Life Technologies; #GAS003) for intracellular staining to detect IFNg (Biolegend; #502532) (Singh et.al., Oncoimmunology. 2016; 5(1): e1040216) using flowcytometry.

Moreover, T-cells from each treatment group were stained for CAR-19 expression using Affinpure goat-anti mouse IgG (Jackson ImmunoResearch, West Grove, PA, USA, Cat# 115-065-072) diluted in FACS buffer. Furthermore, surface phenotype (CCR7, CD62L, CD95, CD45RO) was determined using standard flow cytometry protocol as previously described to detect the t-cell subtypes in each group.

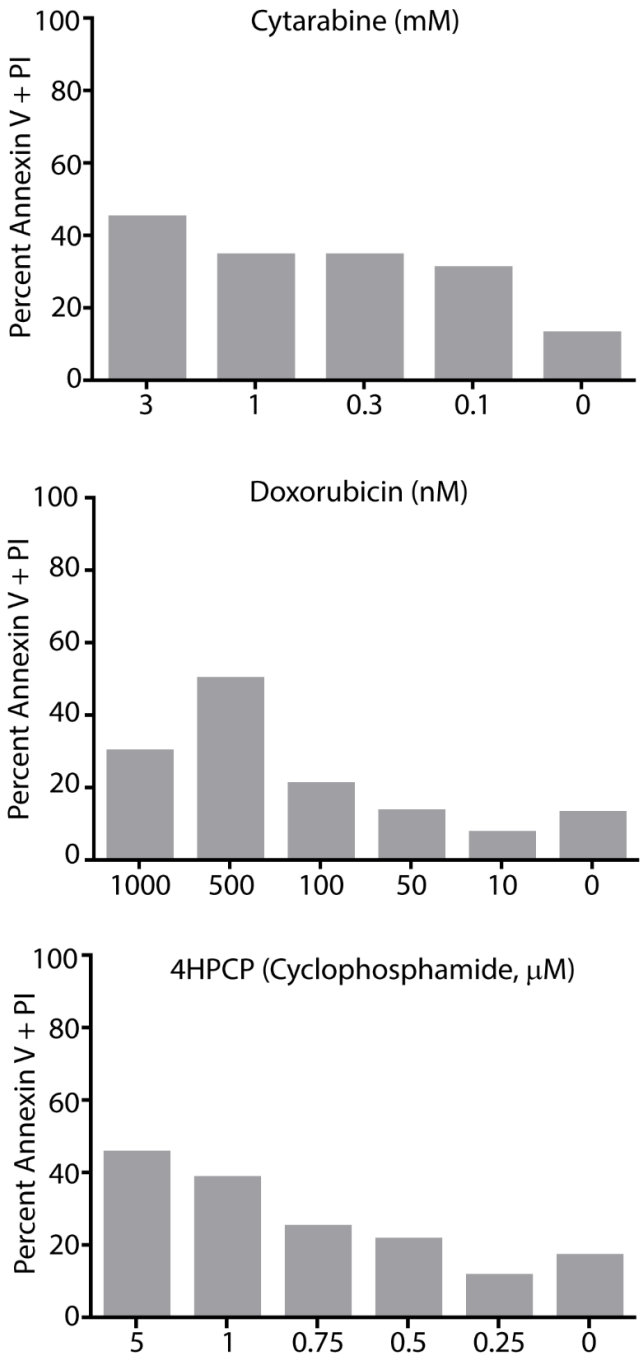

Figure S1: T cells were exposed to short term chemotherapy and allowed to recover as in the primary methods. Doses were based on literature searches on in vitro anti-tumor effects and extended one half log10 in each direction. Percent of apoptotic cells is represented, with dose response seen with each agent though IC/LD50 not reached for mature T cells for any agent. As the doses of these agents have establishe human toxicity correlates, we did not pursue hgher dosing as we did not feel it relevant to our model.

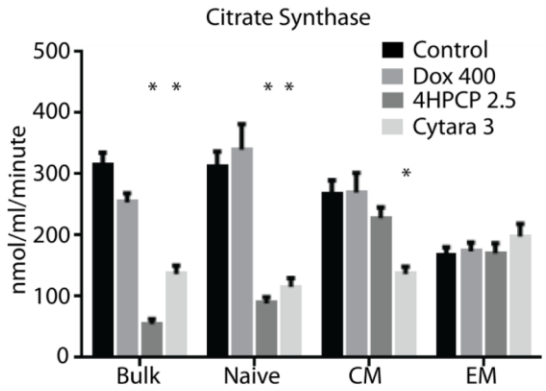

Figure S2. Citrate Synthase activity in T cells exposed to chemotherapy (short term). T cells were sorted into the indicated subsets and exposed to chemotherapy for 24 hours then allowed to recover. This mitochondrial enzyme is reduced in cyclophosphamide and cytarabine exposed Naïve cells but relatively preserved in memory cells. EM cells seem resistant to any change. *=$p<0.01$

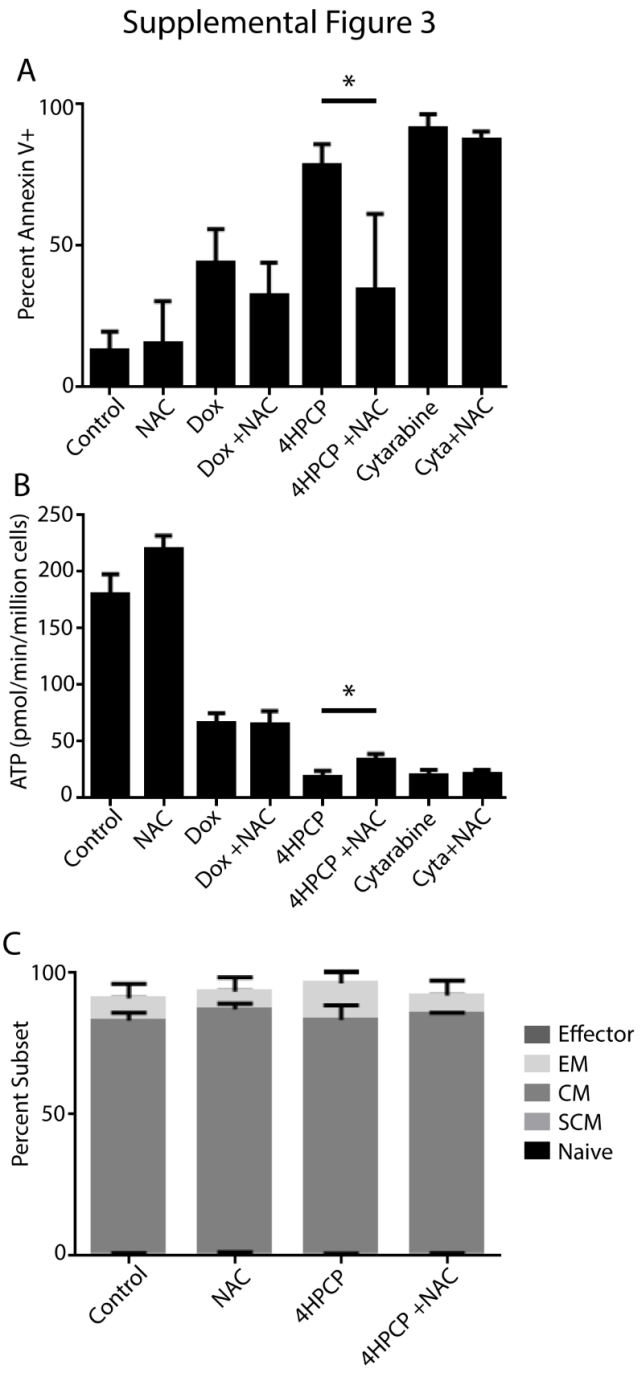

Figure S3: T cells were exposed to chemotherapy and allowed to recover as in Figure 3. Cells were assessed for apoptosis by Annexin V staining in Panel A, where a significant reduction of apoptotic cells was only seen with cyclophosphamide. In addition, with the same treatment schema, T cells were assessed for mitochondrial ATP production (another readout of the Seahorse analysis in Figure 3). Panel B shows that ATP production is down in all chemotherapy treated cells but only cyclophosphamide treatment had in a significant recovery of ATP with NAC though still well below untreated cells. Panel C shows T cells from FIgure 6, demonstrating no significant change is surface phenotype after stimulation regardless of treatement. T cells are predominantly CM with little variability. *=$p<0.05$ SCM = Stem central memory, CM=central memory, EM=effector memory.

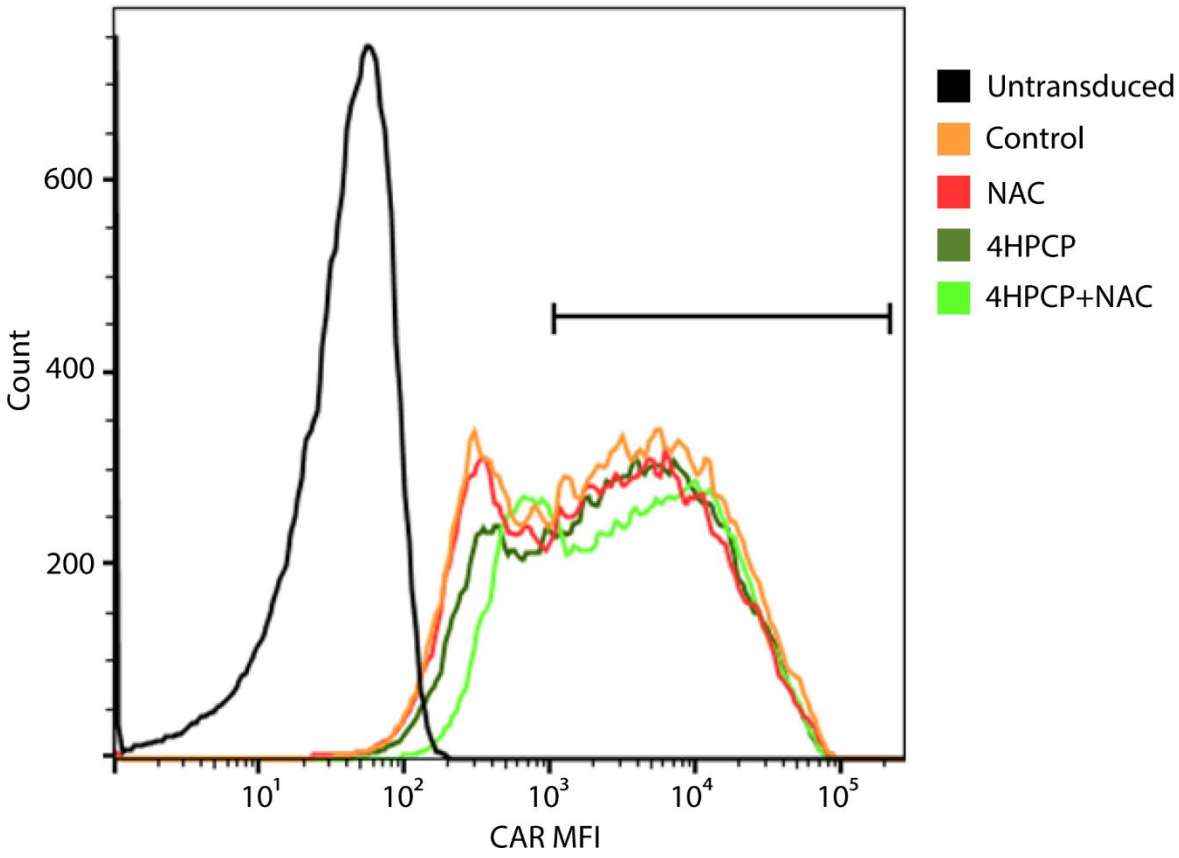

Figure S4: T cells used in Figure 6 stained for the CAR as in supplemental methods. There is no difference in CAR transduction efficiency or MFI between groups.